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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Diago, A.I. de, "The Behavior of the White Rat vis-a-vis B. anthracis," Gaceta Veterinaria, v. 16, no. 91, Sept.-Oct. 1954, Buenos Aires, pp. 204 - 208 (English translation, R. Blair).

Practice has shown that to date there is not available a satisfactory test for the control over laboratory animals of the protective capacity of the anthrax vaccines currently in use. Neither the rabbit, the field mouse, nor the guinea pig have given results useful for general application despite some favorable information on their use.

As far as we, with our modest means, have been able to review the literature, we have not found works in which the white rat was used with this in view. This is natural, bearing in mind that this rodent has always been considered refractory, or at least little receptive, to anthrax infection, since its serum possesses bactericidal powers against B. anthracis (1)

Procedures tending to diminish the body defenses have been used with this animal, as with others displaying similar behavior, in order to obtain a receptivity to the infection. A classic is the experiment of Charrin and Roger, who succeeded in infecting the rats by submitting them to intense work in a rotating cage. Nor in this respect either have we found literature concerning the adaption of a strain to the rat for the purpose of obtaining regularly positive infections.

Therefore, we tried first to adapt a strain of B. anthracis to the rat in order then to see if this animal could be employed in the laboratory to determine the immunizing capacity of the anthrax vaccines.

Materials and Methods

For our work we chose from our collection a strain of B. anthracis designated "15 A," isolated in 1950 from the bone marrow of a calf and vaccinated

some months earlier.

No definite rate, indiscriminately male and female, of an average weight of 150 grams. Each series of inoculations was done on groups of four animals. It was started by inoculating--always subcutaneously--large quantities of culture, 0.5 cc of viable elements in a physiological solution, having an opacity approximating Tube No. 7 of the McFarland Nephelometer, obtained from a 24 hour culture on simple agar with Burke Davis peptone. Always using the same system, successive passages were made isolating the germ from the blood of the heart of the first animal which died, and then inoculating another series of four; and so on, successively. The concentration of the suspension was gradually reduced until an LD₅₀ was obtained by injecting 0.5 cc of a suspension which contained an average of about 350,000 viable elements in all. This was obtained from the 26th passage in rats, which it killed in from 48 to 96 hours.

The control of the viable elements (spores and vegetative cells) was effected by a sheep's blood agar plate count, seeding 0.1 cc of a 1/1000 solution of the suspension to be injected.

Once we had obtained the adapted strain of constant pathogenic action on the rat, we proceeded to the second phase, that of determining whether the rat was suitable for measuring the immunizing property of anthrax vaccines.

We took 25 rats of 200 grams weight and divided them into 6 lots. Three lots we inoculated subcutaneously with 0.5 cc of sporulated vaccine consisting of a suspension in physiological solution of 1,500,000 spores per cc of our strain "C" vaccine, repeating the dose after 18 days. After nine days of the second dose we inoculated these--as well as the three control lots--with one, two, and four LD₅₀ respectively of the rat-adapted strain.

Results

The results may be seen in Figure 1, from which it is apparent that the double vaccination with Strain "C" did not succeed in inducing any defense in the rats. Rather, the contrary would appear to be the case.

On the other hand the regular pathogenicity of the rat-adapted strain is evident.

Figure 1

Twenty-nine rats were vaccinated twice at 18 days interval against anthrax. Nine days after the second dose, along with the 20 controls, they were administered the virulent test dose.

10 vaccinated rats inoculated with 1 LD ₅₀	1 died after two days; 3 after 4 days; 6 survived
10 unvaccinated control rats inoculated with 1 LD ₅₀	10 survived
10 vaccinated rats inoculated with 2 LD ₅₀	5 died after 3 days; 1 after 4 days; 4 survived
10 unvaccinated control rats inoculated with 2 LD ₅₀	2 died after 3 days; 8 survived
9 vaccinated rats inoculated with 4 LD ₅₀	3 died in 24 hours; 4 in 2 days; 1 in 4 days; 1 in 5 days; 1 in 7 days
9 unvaccinated control rats inoculated with 4 LD ₅₀	6 died in 2 days; 2 in 4 days; 1 survived

Discussion

There are some works which report favorable results on the immunization of laboratory animals with B. anthracis; and others, on the contrary, which have not succeeded. It is understood that we refer only to the use of spores or bacillary forms of B. anthracis as an antigen, and not other special antigens. Thus, Staub (1949) (2) mentions the "Waybridge" strain of Gladstone which "is sporogenous, avirulent, capsulated, and is immunogenic for sheep, the rabbit, the guinea pig, but not for the field mouse."

Sterne (1939) (3) affirms that guinea pigs and rabbits can be immunized without difficulty against anthrax. Utilizing guinea pigs in routine tests for the antigenic control of their vaccine (5), it is found that they already have a certain degree of protection 24 hours after vaccination, and are still protected after 6 months (4). He also succeeded in protecting field mice, although to a lesser degree than guinea pigs. He found differences in the behavior of different strains.

Torres Muñoz (1950) (6) also succeeded in vaccinating guinea pigs, but only by using the colonies in Phase II of some of his strains.

Wellman and Berdich (1933) (7) vaccinated rabbits intradermally with Pasteur vaccines 1 and 2.

On the contrary, Boguit and Stenz (1930) (8) and especially Basset (9) did not succeed.

One could go on citing many other works in one sense or the other, but from a study of them it is clear that the disparity of results is due fundamentally to the vaccine strain used, as Pacharn (1950) (10) believes

¹While the present article was in press we were informed that the "Waybridge" strain of Gladstone is the same as the vaccine of Sterne.

is possible.

However, it is quite probable that, besides the factor of the strain, there is another cause, since we must not forget that Pasteur (11) invariably succeeded in vaccinating the guinea pig with his anthrax vaccines 1 and 2.

Nor in our work have we succeeded in protecting the rats by using Strain "G"; even though we should succeed in vaccinating them with other strains, these would not serve for general use for this purpose.

Results

One strain of B. anthracis was adapted to the white rat, obtaining an LD₅₀ of 350,000 viable elements after 26 passages.

Vaccinated rats showed no protection against one LD₅₀ of the rat-adapted strain.

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